Appln. No. 10/735,335
Amendment dated November 30, 2004
Reply to Office Action of October 29, 2004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Doddabele L. Madhavi, *et al.*
Serial No.	:	10/735,335
Filed:	:	December 12, 2003
For:	:	Bioavailable Carotenoid-Cyclodextrin Formulations For Soft-Gels And Other Encapsulation Systems
TC/AU	:	1623
Examiner	:	Matthew L. Fedowitz
Attorney Docket No.	:	BIO 2-016

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DECLARATION UNDER C.F.R. § 1.132

Doddabele Madhavi, does declare and state that:

1. She received a Bachelor of Science degree in Biology and Chemistry in 1977 from University of Mysore, Mysore, India; a Master of Science degree in Botony in 1979 from Mysore, Mysore, India, Majors: Plant Biochemistry, Physiology, and Genetics; and a Doctorate degree (Ph.D.) in Biochemistry in 1987 from Central Food Technological Research Institute, Mysore, India, Thesis : Studies on the effects of processing on amino acid availability and functional properties of vegetable proteins.
2. She was a Research Fellow at the Department of Protein Technology, Central Food Technological Research Institute, Research Focus: Physico-chemical properties of vegetable proteins. Mysore, India, '80 to '87; a Research Scientist at the Department of Fruit and Vegetable Technology, Central Food Technological Research Institute. Research focus: Phytochemicals in cell cultures of food crops. Mysore, India, '88 to '90; a Research Associate at the Department of Nutrition and Food Sciences, Utah State University. Research focus: Color stability in bovine skeletal muscles. Logan, UT, '91 to '93; a Research Associate at the Departments of Food Science and Horticulture, University of Illinois, Research focus: the presence and use of flavonoids in crop plants and cell cultures. Urbana, IL, '93 to '96; a Visiting Assistant Professor at the Department of Natural Resources and Environmental Sciences, University of Illinois, where she researched the screening, extraction and formulation of bioactive compounds from novel sources. Urbana, IL, '96 to 98; Chief Scientist for PhytoLife Sciences, Inc. where she

provided a mid-course evaluation of a novel technology to produce natural product for a company in a turn-around situation. With management, developed enhancements and alternatives to the technology to meet pressing needs to generate revenues. Succeeded in redirecting the Company's conceptual basis while still maintaining its core value. Responsible for all laboratory functions Columbus, OH, '98 to '99; and currently is Managing Partner for BioActives LLC, the assignee of the above-identified application where she provides the scientific expertise for new product development, process development and scientific/experimental strategies therein. In collaboration with management chart the company's strategic direction. Responsible for all laboratory functions including but not limited to the concept, experimental design and execution, ordering equipment and supplies, and personnel and environmental safety. Worcester, MA. Oct. '99 to present.

3. A more complete resume for her is attached hereto.
4. She is a co-inventor of and co-applicant for the invention described and claimed in the above-identified application.
5. She has read an Office action dated October 29, 2004 and the art cited therein and as applied in a rejection of the claims.
6. It is well known in the field of cyclodextrins that formation of inclusion complexes and improvement in properties, such as bioavailability and solubility of the active molecule, is highly variable and unpredictable. It also is known that the different cyclodextrins used in the current invention have different properties in terms of cavity size, hydrophobicity, and molecular weight. They interact differently with the same active hydrophobic molecule, are stereo-selective, and some may form the inclusion complex while others may not interact.
7. The following is a list of some of the publications, which highlight the differences in properties of inclusion complexes of different cyclodextrins with specific pharmaceuticals and natural compounds: Alexander, J.M., Clark, J.L., Brett, T.J., Stezowski, J.J., "Chiral discrimination of cyclodextrin complexes of amino acid derivatives: beta-cyclodextrin/n-acetyl-L-phenylalanine and N-acetyl-D-phenylalanine complexes", *Proc. Natl. Acad. Sci., USA*, 99: 5115-20, 2002; Mura, P., Faucci, M.T., and Bramanti, G., "Influence of the preparation method on the physicochemical properties of binary systems of econazole with cyclodextrins", *Int. J. Pharm.*, 193: 85-95, 1999; Zia, V., Rajewski, R.A., and Stella, V.J., "Effect of cyclodextrin charge on complexation of neutral and charged substrates: comparison of (SBE)7M-beta-CD to Hp-beta-CD", *Pharm. Res.*, 18: 667-73, 2001; Tannesen, H.H., Hanne, H.T., Masson, M., and Loftsson, T.,

"Studies on curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility and photochemical stability", *Int. J. Pharm.*, 244: 127-135, 2002; Miyake, K., Arima, H., Hirayama, F., Yamamoto, M., Horikawa, T., Sumkiyoshi, H., Noda, S., and Uekama, K., "Improvement of solubility and oral bioavailability of rutin by complexation with 2-hydroxypropyl-beta-cyclodextrin", *Pharm. Dev. Technol.*, 5: 399-407, 2000; Vakily, M., Pasutto, F.M., Daneshtalab, M., and Jamali, F., "Inclusion complexation of heptakis(2,6-di-O-ethyl)-beta-cyclodextrin with tiaprofenic acid: pharmacokinetic consequences of a pH-dependent release and stereo-selective dissolution", *J. Pharm. Sci.*, 84: 1014-9, 1995; Mura, P., Adragna, E., Rabasco, A.M., Moyano, J.R., Perez-Mertinez, J.I., Arias, M.J., and Gines, J.M., "Effects of host cavity size and the preparation method on the physicochemical properties of ibuproxam-cyclodextrin systems", *Drug Dev. Ind. Pharm.*, 25: 279-87, 1999.

8. Carotenoids are broadly classified as hydrocarbon carotenoids and xanthophylls. It is well known that carotenoids vary in their molecular structure, have stereoisomers, and some are more hydrophobic than others. For example, the parent 40 carbon atoms are in an open ringed, highly unsaturated configuration with lycopene, while in lutein or zeaxanthin there is cyclization at both the ends of the polyene chain and substitution of oxygen containing groups. Apocarotenoids generally have fewer than 40 carbon atoms in their structure. Because of changes in structure and hydrophobicity, the carotenoids in general show different affinities towards cyclodextrins.

9. The hydrocarbon carotenoids, β-carotene and lycopene, are less polar as compared to lutein and zeaxanthin, for example, which may differ in their affinity towards cyclodextrins. Even within the same class, for example xanthophylls, lutein and zeaxanthin are stereoisomers, which may have different affinities for a cyclodextrin. It has also been reported that complexation of astaxanthin, another xanthophyll, with sulfobutyl ether beta-cyclodextrin does not improve the solubility to result in a pharmaceutically acceptable chemical delivery system for humans (Lockwood, S.F., O'Mailey, S., and Mosher, G.L., "Improved aqueous solubility of crystalline astaxanthin by Captisol", *J. Pharm Sci.*, 92: 922-6, 2003).

10. Complexation with cyclodextrins often may <u>not</u> result in increased bioavailbility. For example, according to Spirichev *et al.* (1996) uptake of β-carotene from a cyclodextrin complex was lower as compared to the commercial oil dispersions or microencapsulated beadlets in human studies (Spirichev, V.B., Iakushina, L.A., Isaeva, V.A., Shkarina, T.N., Malakhova, E.A., and Poznanskaia, A.A., "Study of bioavailability of different forms of synthetic beta-carotene in volunteers", *Vopr. Pitan.*, 6: 22-6, 1996; article in Russian).

11. Hence, it is not obvious from the teachings of the art cited against the claims [Leuenberger (U.S. Patent No. 5,221,735), Fukamachi (U.S. Patent No. 4,929,774), Patel (U.S. Patent No. 6,569,463), and Orthoefer (U.S. Patent No. 4,125,630)] that different carotenoids can be complexed with the natural cyclodextrins or their derivatives or that complexation in general improves bioavailability. It also is not obvious that a mixture of stereoisomers can be complexed in a manner resulting in simultaneous uptake of the isomers into the cells. Further such teachings do not indicate the variability in uptake based on the cyclodextrins, a factor important for feasibility of commercial production and application of the complex.

12. The present invention describes a practical commercial process for making carotenoid/cyclodextrin molecular inclusion complex and suitable formulation excipients. The inclusion complexes contain a minimum of 20% carotenoids and the process is highly efficient with at least 95% recovery. Leuenberger, for example, uses weight ratios of the carotenoids and cyclodextrin for complexation and the ratio is preferably about 1:10 to about 1:200. In the present invention, the inventors use molar ratios for making the complexes, with the molar ratio of carotenoid and cyclodextrin between 1:0.5 to 1:10. The molar weight ratios result in a predictable end product suitable for commercial production.

13. The teachings by Leuenberger result in very dilute solutions of lycopene/apocarotenol complexes with methyl β-cyclodextrin, β-cyclodextrin, and α-cyclodextrin (2 μg lycopene/ml, 0.16mg/ml apocarotenol). It is <u>not</u> obvious from the data that a commercially feasible process can be developed. The limits of this teaching would make it difficult, if not impossible, to incorporate a daily dosage of lycopene in a nutritional supplement capsule or fortified food product. Furthermore, the relative costs of such a product at most of the range would be greater than the related bioavailability gained, and thereby produce a less effective product as compared to the original ingredient.

14. The teachings by Leuenberger do not indicate whether lycopene/apocarotenol, or other carotenoids, like lutein or zeaxanthin, can be complexed with different cyclodextrins, or such complexes can be made on a commercial scale, or formulated to retain their properties. Since the cost of cyclodextrins vary, such information is crucial to developing a cost-effective product, especially in a cost-conscious nutritional supplement industry.

15. The carotenoid/cyclodextrin complex disclosed in the above-identified application is <u>not</u> water-soluble. Leuenberger describes that the bioavailability improves when the carotenoids become soluble in polar solvents, especially water.

16. The excipients used in the present invention are well known in the art of making formulations for soft gelatin capsules and other soft-shell encapsulations, both in the supplement and pharmaceutical industry.

17. Leuenberger describes use of oil to dissolve/disperse carotenoids followed by emulsification with water. Fukamachi describes use of vegetable oils in microencapsulation formulations for oxidation sensitive compounds and mentions lutein and zeaxanthin. However, the oils are used again for making an emulsion with the gelatin matrix, an application entirely different from using the oil as an excipient or filler for the cyclodextrin complex, as in the present invention. Orthoefer teaches using triglycerides as plasticizers for making meat analogs from vegetable proteins, again an application entirely different from formulating a carotenoid-cyclodextrin complex into a dosage form as in the present invention. Patel teaches the use of surfactants in the formulation. Again, it is not obvious from these teachings whether a carotenoid cyclodextrin complex can be formulated w ith these excipients without any adverse effects on the stability of the complex or the bioavailability for at least the reasons detailed below.

18. It is well known in the carotenoid art that the cyclodextrin form dynamic, non-covalent inclusion complexes and the weak bonds can be disrupted by a number of factors, including, *inter alia*, excipients used in formulations. The complexes are sensitive to commonly used excipients in dosage formulations, such as, for example, vegetable oils, medium chain triglycerides, and synthetic surfactants such as polysorbates, polyethylene glycols, and phospholipids such as lecithin. The excipients with different polarities may interact with cyclodextrins resulting in the dissociation of the complex, inhibit the release of the actives, or modulate the dissolution properties. The interactions in general are often unpredictable.

19. The following is a list of some of the publications on the interactions of cyclodextrin inclusion complexes of pharmaceuticals and flavor compounds with formulation excipients: Trinh, T., "Non-destructive carriers for cyclodextrin complexes", U.S. Patent No. 5,384,186; Veiga, M.D., and Ahsan, F., "Influence of surfactants (present in the dissolution media) on the release behaviour of tolbutamide from its inclusion complex with beta-cyclodextrin", *Eur. J. Pharm. Sci.*, 9: 291-9, 2000; Ahsan, F., Arnold, J.J., Meezan, E., and Pillioin, D.J., "Mutual inhibition of insulin absorption-enhancing properties of dodecylmaltoside and dimethyl-beta-cyclodextrin following nasal administration", *Pharm. Res.*, 18; 608-614, 2001; Shalko-Basnet, N., Pavelic, Z., and Becirevic-Lacan, M., "Liposomes containing drug and cyclodextrin prepared by the one-step spray-drying method", *Drug Dev. Ind. Pharm.*, 26: 1279-84, 2000; Valero, M., Corillo, C., and Rodriguez,

L.J., "Ternary naproxen: beta-cyclodextrin: polyethylene glycol complex formation", *Int. J. Pharm.*, 265: 141-9, 2003; Yu, S.C., Bochot, A., Bas, G.L., Cheron, M., Mahuteau, J., Grossiord, J.L., Seiller, M., and Duchene, D., "Effect of camphor/cyclodextrin complexation on the stability of O/W/O multiple emulsions", *Int. J. Pharm.*, 261: 1-8, 2003.

20. The drying methods tested in the examples of the above-identified application included freeze-drying and spray-drying, which both are well known in the art of commercial process development for supplements, pharmaceuticals, and food products. The invention describes a commercially efficient process, which includes freeze-drying an aqueous dispersion of carotenoid-cyclodextrin complex. Freeze-drying was found to be efficient as compared to spray-drying with a 95% recovery of the product, as compared to 50% loss with spray-drying. Further, to our surprise, the freeze-dried product was superior to spray dried product in bioavailability studies.

21. Fukamachi teaches that a formulation for microencapsulation of oxidation sensitive compounds can be freeze-dried. It is not obvious from these teachings that freeze-drying is a better commercial method as compared to spray-drying for a carotenoid-cyclodextrin complex or that freeze-drying results in an improved product as compared to spray-drying.

22. It is well known in the art that hydrophobic compounds present delivery challenges because of their physicochemical properties and soft gelatin capsules may offer a delivery system. However, complexation of carotenoids with cyclodextrins in general resulted in a hydrophilic, water dispersible fine powder. Such complexes are used for making directly compressible tablets or incorporated in to hard gelatin capsules, as cyclodextrins are expected to stabilize sensitive compounds against degradation. However, we found that complexation with cyclodextrins did <u>not</u> stabilize the carotenoids to afford the necessary commercially accepted shelf life in tablets or hard capsules. The soft-gelatin formulation was developed to stabilize the carotenoids.

23. When hydrophobic excipients, such as vegetable oils, are used, they may inhibit the dispersion of the complex in water; thus, reducing the uptake of the active molecule. However, to our surprise, we found that the complex retained its properties even after formulation with vegetable oil or vegetable oil-lecithin as excipients.

24. Thus, it is not obvious from Patel that the hydrophilic carotenoid-cylodextrin complex can be stabilized or made bioavailable by incorporation in to soft gelatin capsules as delivery system.

25. Thus, in her opinion, it was totally unexpected that a commercially feasible, practical, and commercially viable process resulted for making a bioavailable cyclodextrin/carotenoid

complex by freeze-drying a cyclodextrin/carotenoid complex in a molar ratio of between about 0.5:1 and 10:1, and adding such freeze-dried complex to a vegetable oil. The art cited simply does not render obvious the present invention.

26. All statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

FURTHER DECLARANT SAYETH NAUGHT.

Date 12/7/'04

Dr. Madhavi.
Doddabele Madhavi, Ph.D.